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CO_2-induced kidney calcification

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Schaefer, K. E., S. M. Pasquale, A. A. Messier, and H. Niemoeller. 1979. CO_2-induced kidney calcification. Undersea Biomed. Res. Sub. Suppl.: S143–S153.—Light microscopic examination of kidney tissue of guinea pigs exposed to 1.5% CO_2, 21% O_2, and balance N_2 for periods as long as 42 days and of rats exposed to the same CO_2 concentrations for up to 91 days showed that the incidence of focal kidney calcification increased with length of exposure. Calcification occurred primarily in the tubules of the renal cortex. Another group of guinea pigs were exposed to 1% CO_2, 21% O_2, and the balance N_2 for periods up to six weeks and were later killed at regular intervals, together with control animals of the same litter. In the exposed animals, arterial P_{CO_2} was elevated by 3–4 mmHg and hydrogen ions by about 4 nmol/liter. The standard bicarbonate level was lowered by 1–1.5 mmol, indicating a lack of renal reabsorption of bicarbonate (HCO_3), which in turn placed greater stress on the bone buffer system and apparently caused bone calcium and phosphorus mobilization. Bone calcium and phosphorus levels exhibited a cyclic decrease, which resulted in cyclic hypercalcemia and hyperphosphatemia, after one week and six weeks of exposure to 1% CO_2. Kidney calcium content increased significantly after two weeks of exposure (27%) and remained at this elevated level during subsequent exposures between the third and sixth weeks. These findings indicate that once the kidney calcification process has started, kidney mineralization is independent of fluctuations in the blood calcium level. A rise in plasma phosphate level that occurred after one day of exposure could have been a precipitating factor in the calcification process. The small but consistent increases in ionized calcium during a 4-week exposure to 1% CO_2 may have stimulated the parathyroid, causing an increased blood calcium level that was independent of the two calcium tides in the blood associated with marked bone calcium loss.

chronic CO_2 toxicity
calcium and phosphorus metabolism
metabolic acidosis
kidney calcification

Chronic CO_2 studies in men exposed to 1.5% CO_2 for prolonged periods demonstrated changes in calcium and phosphorus metabolism that appeared to be associated with storage and release of CO_2 from the bones (Schaefer, Nichols, and Carey 1963). One of the main findings of histopathological investigations of animals exposed to 3% and higher CO_2 concentrations was kidney calcification (Meesen 1948; Zinck 1949). In subsequent combined physiological and histopathological studies of guinea pigs exposed to 15% CO_2 for prolonged periods,

significant renal calcification was observed, consisting of interstitial calcium deposits in the renal medulla, and calcium deposits in the tubules and in the basement membranes of the renal cortex (Schaefer, Hasson, and Niemoeller 1961). Significant changes in calcium metabolism observed in American and British submarine patrol studies (Gray, Morris, and Brooks 1973; Messier, Heyder, Braithwaite, McCluggage, Peck, and Schaefer 1979) focused attention on the need for further exploration of the relationship between CO_2-induced changes in calcium metabolism and kidney calcification during prolonged exposure to low levels of CO_2 (in the range between 1% and 1.5%). Results of such studies are presented in this report.

MATERIALS AND METHODS

Experiments were carried out with mature male guinea pigs of the Hartley strain, weighing between 400 and 600 grams (g), and mature male rats, between 75 and 120 days of age, from the Harvard Biological Laboratory and from the Charles River Laboratory. In the first series of experiments carried out in 1961, guinea pigs and rats were exposed to 1.5% CO_2, 21% O_2, and the balance N_2. The gas mixtures were obtained by mixing 100% CO_2, supplied from a bank of cylinders charged to 1800 psi, with air delivered to the gas inlet from high-pressure oxygen cylinders. Animals were killed at different periods of exposure to 1.5% CO_2 and tissues were examined with the light microscope. The exposure chambers used in this series had a water-cooled temperature control system and a closed-circuit air ventilation system that continuously circulated chamber air through silicagel containers. Under these conditions, the temperature in the exposure chambers was kept at 25.6 ± 1.1°C and the humidity between 65 and 75%. Ammonia vapor was absorbed by boric acid spread on the floor of the cages. The carbon dioxide level in the chambers was continuously monitored with a Beckman infrared CO_2 meter, and the oxygen content with a Beckman E 2 Oxygen Analyzer. An automatic gas sampling device switched from one exposure chamber to another every 20 min, and recordings were made of gas concentrations.

In the second series of experiments, guinea pigs were exposed to 1% CO_2, 21% O_2, and the balance N_2 in commercially built environmental control chambers with automatic temperature and humidity controls. The environmental temperature was kept at 25.6 ± 1.1°C, and the humidity between 65 and 75%. The gas mixtures were prepared by mixing proportional amounts of CO_2 to air; oxygen was added from a high-pressure cylinder. The air within the chamber was recirculated 12 times a minute. With this fast and large turnover of chamber air, mixing of CO_2 and air was nearly instantaneous. The carbon dioxide concentration in the chamber was continuously monitored with a Beckman infrared CO_2 analyzer, and the oxygen content was sampled intermittently with a Beckman O_2 analyzer. The CO_2 concentrations were kept at 1% within limits of ± 0.1%, and the oxygen concentrations at 21% ± 0.5%. Ammonia vapor was absorbed by boric acid placed in the chamber. The chamber was opened each morning for a period of 3–5 min to fill the water and food containers and to remove urine and feces.

Litter mates of the animals exposed to CO_2 were kept in a second environmental chamber under identical environmental conditions, except that the ambient air was free of CO_2. From the first to the fourth week of exposure, six animals were killed weekly, together with 3–4 control animals. After six weeks of exposure to 1% CO_2, the same procedure was used. During the period of six weeks, the weight of both control and exposed animals increased in the same fashion (Fig. 2). For blood gas studies, 8 control animals were usually used to obtain enough arterial blood samples in which the oxygen partial pressure was above 50 mmHg, the criterion set in our laboratory for acceptable values.

Prior to blood sampling, the animals received 40 mg pentobarbital/kg body weight (wt) subcutaneously and were returned to the CO_2 exposure chamber. The anesthesia was usually effective after approximately 5 min, at which time the animals were taken out of the exposure chamber and placed immediately under a mask, through which they breathed the same CO_2 gas mixtures to which they had been exposed. Blood samples were drawn from the abdominal aorta. Blood pH and Pco_2 were determined with an Instrumentation Laboratory blood gas and pH analyzing system. The femurs of both legs were removed and rapidly cleaned and stripped free of adhering tissues and bone marrow. Compact bone specimens (between 200–300 mg) were kept on ice for determination of total CO_2 content and bone electrolytes. The time between procurement and analysis of the fresh samples did not exceed two hours. Paired specimens were oven-dried to constant weight at 150°C for 18 h before analysis.

Bone and kidney calcium were determined with an atomic absorption spectrometer. For calcium determination both bone and kidney samples were dry-ashed at 650°C for 24 h. Appropriate dilutions with 3 N hydrochloric acid were used for analysis; recovery was assessed by adding known amounts of calcium.

Phosphorus of bone samples was measured on acid digests of bone. Bone samples were dissolved in 6 N hydrochloric acid, and the appropriate dilution was analyzed according to a method modified from that of Fiske and Subbarow (1925).

In a separate preliminary experiment, guinea pigs of the Hartley strain weighing between 400 and 600 grams were exposed to 1% CO_2 for 4 weeks. Acid-base parameters and blood electrolytes were measured, using the methods listed above. However, bone specimens were not analyzed. Ionized calcium was determined using an Orion Model 99-20 sensor calcium flow-through system connected to an Orion Model 801 Digital pH/mV meter for determination of serum ionized calcium. Blood samples for the measurement of ionized calcium were withdrawn by syringe and quickly injected to fill completely a Vacutainer containing no anticoagulant.

Tissue specimens (kidneys) from the first group of experimental animals were fixed in buffered formalin, embedded in paraffin, sectioned and routinely stained with hematoxylin and eosin. In certain cases Masson stain was also used. A card was prepared for each animal on which the results of the histological examination of the various organs were entered. After this, another evaluation was made comparing the findings of individual organs in a whole experimental series. The principal histological changes were graded from 1 to 3 and charted. In the second experimental series, no histopathological investigation of the kidneys was performed. All statistical comparisons of physiological data were done by Student's *t*-test.

RESULTS

Table 1 shows the results of histopathological studies of the kidney carried out in guinea pigs and rats exposed to 1.5% CO_2 for prolonged periods. No significant changes were observed in kidney morphology, except in renal calcification. Incidence of focal and tubular kidney calcifications increased with the duration of exposure in both guinea pigs and rats. During the first two weeks of exposure to 1.5% CO_2, focal kidney calcification was not observed.

Data on blood Pco_2, hydrogen ion concentration, and standard bicarbonate in guinea pigs exposed to 1% CO_2, 21% O_2, and balance N_2 for up to six weeks and those of control animals are shown in Fig. 1. These acid-base parameters did not change in the control animals; however, in the exposed animals Pa_{CO_2} was persistently elevated by 3–4 mmHg and was statistically different from that of controls after 3 and 4 weeks of exposure. Hydrogen ion concentrations of the exposed animals were on the average about 3–4 nmols higher (statisti-

TABLE 1
EFFECT OF PROLONGED EXPOSURE TO 1.5% CO_2 ON KIDNEY MORPHOLOGY OF GUINEA PIGS AND RATS

Conditions	No. of Animals	Focal Calcification, Incidence	Grade
		Guinea Pigs	
Controls	5	0	0
(1.5% CO_2 in 21% O_2)			
1–25 days	6	33%	1
35–42 days	6	66%	1
Recovery on Air			
27 days after			
42 days of exposure	6	66%	1–2
		Rats	
Controls	5	0	0
(1.5% CO_2 in 21% O_2)			
1–15 days	5	0	0
35 days	6	50%	1.0
91 days	5	100%	2.0

cally significant after 1, 2, and 4 weeks). Standard bicarbonate was 1–1.5 mmol lower in exposed animals (statistically significant after 1, 2, and 4 weeks).

Figure 2 shows changes in body weight and in the calcium content of bone, kidney, and plasma in animals exposed for six weeks to 1% CO_2 and in control animals. Body weight increases were virtually the same in control and exposed groups. Bone calcium content in control animals remained at the same level. Animals exposed to 1% CO_2 showed a significant initial fall of bone calcium at the end of one week, followed by a rise to near normal levels after three weeks and a subsequent greater decrease after six weeks of exposure. Blood calcium reflects the changes in bone calcium, rising when bone calcium falls. Blood calcium in control animals tended to be lower than that in exposed animals, and remained at the same level throughout exposure. The kidney calcium content did not change in control animals; however, in the exposed animals it rose slightly after one week and continued to increase after two and three weeks, at which time the kidney calcium content was 27% above control level.

Carbon dioxide-induced alterations in phosphorus metabolism are shown in Fig. 3. Changes in phosphorous levels in bone and blood mirrored those in calcium metabolism. In addition to the phases related to bone phosphorous changes, plasma phosphorus showed a significant increase after the first day of exposure.

Table 2 shows data on ionized calcium measured in a separate experiment in which guinea pigs were exposed to 1% CO_2 for 4 weeks. A slight acidosis occurred, shown by the small increase in H^+ ion concentration. Ionized calcium tended to increase but the changes were not statistically significant.

DISCUSSION

Light microscopic histopathological investigations were carried out in 1961 by Dr. Niemoel-

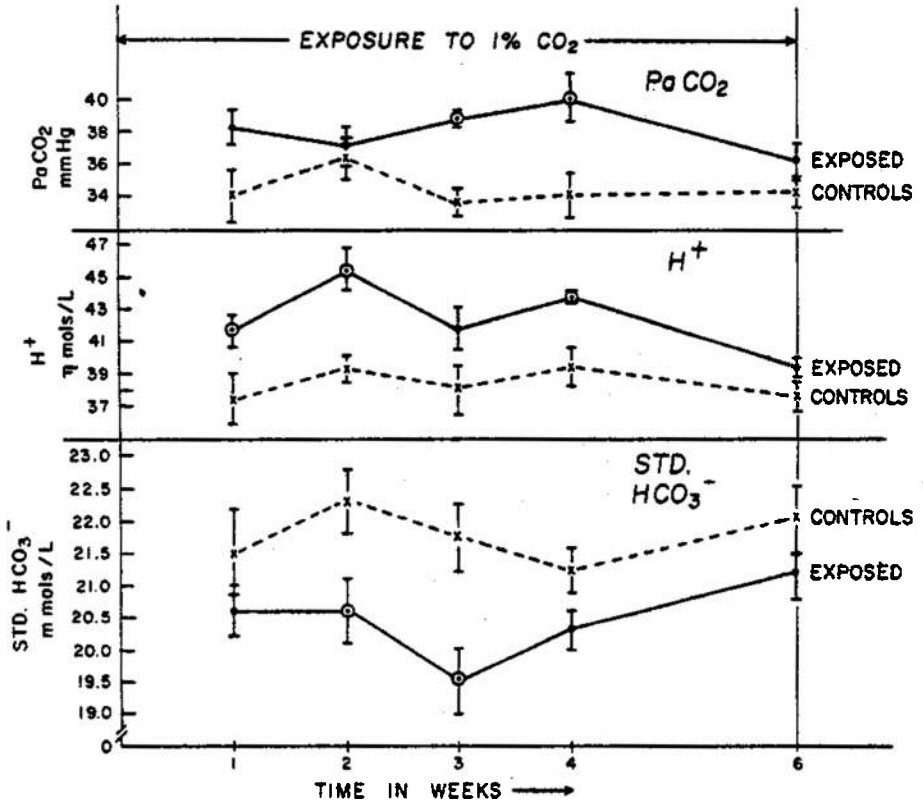

Fig. 1. Effect of prolonged exposure to 1% CO_2 on arterial P_{CO_2} (Pa_{CO_2}), hydrogen ion concentrations (H^+), and standard bicarbonate. Data represent means ± SEM. Significantly different from controls at the 5% level or better. Each exposed group consisted of 6 animals; each control group (litter mates of exposed guinea pigs) consisted of 7–8 animals in blood gas studies.

ler at the Department of Pathology, Yale University, as part of a larger CO_2 project. Guinea pigs and rats exposed for prolonged periods to 1.5% CO_2 showed focal tubular calcification that increased with the length of exposure. The pattern of renal calcification observed during prolonged exposure to 1.5% CO_2 was similar to that seen during exposure to higher CO_2 concentrations (Schaefer et al. 1961). The types of calcification included interstitial deposits, which were generally confined to the medulla, intratubular deposits, and, occasionally, calcification in the tubular basement membranes in the cortex and medulla close to the corticomedullary junction. The pattern of CO_2-induced renal calcification reported here is consistent with that observed under other conditions, e.g., magnesium deficiency, where calcification was reported usually to occur within the lumen of the renal tubules at the junction between the medulla and cortex (Schneeberg and Morrison 1965; Meyer and Forbes 1967; Hamuro, Shino, and Suzuoki 1970).

Prolonged exposure to 1% CO_2 produced a metabolic acidosis, indicated by the increase in H^+ associated with a fall in standard bicarbonate. This indicates that the kidney does not respond to the CO_2-induced acidosis by increasing renal bicarbonate reabsorption, which is the typical response in hypercapnia caused by higher CO_2 concentrations (Schwartz, Brackett, and Cohen 1965). The acidosis produced by prolonged exposure to 1% CO_2 should therefore be considered a predominantly metabolic acidosis. Under these conditions, kidney-calcium

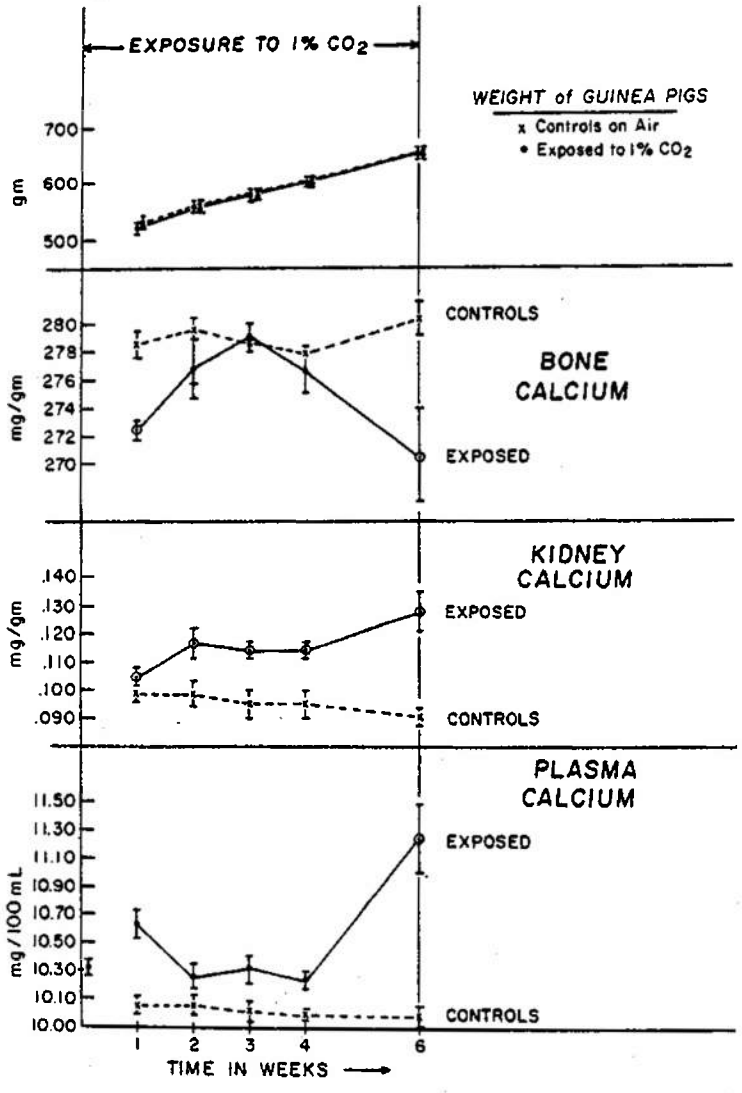

Fig. 2. Effect of prolonged exposure to 1% CO_2 on body weight, bone calcium, kidney calcium, and plasma calcium in guinea pigs. Data represent means ± SEM. ⊙ = significantly different from controls at the 5% level or better. Each experimental group consisted of 6 animals, each control group of 3–4 animals.

content increased significantly after the second week of exposure. Since a 25% rise in kidney calcium content has been accepted as evidence of kidney calcification (Britton and Stokstad 1970), the elevation of kidney calcium found under conditions of 1% CO_2 is evidence of kidney calcification.

Two causes of CO_2-induced kidney calcification have to be considered: (1) hypercalcemia produced by CO_2-induced bone calcium mobilization, with or without involvement of parathyroid hormone stimulation, and (2) hyperphosphatemia. Carbon dioxide uptake in the bones was found to be associated with loss of bone calcium and phosphorus in rats exposed to 8% CO_2 for periods of 2, 4, and 6 weeks (Claudon, Reichart, Bolot, Berstein, and Sabliere 1976). Freeman and Fenn (1953) exposed rats to 10% CO_2 for periods of from 6 to 28 days.

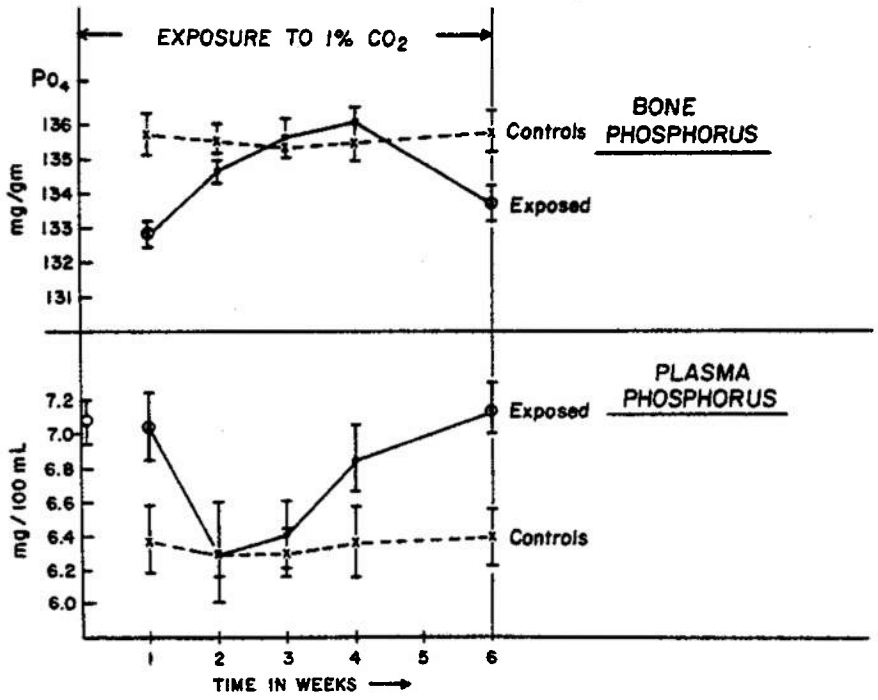

Fig. 3. Effect of prolonged exposure to 1% CO_2 on bone and plasma phosphorus. Data represent means ± SEM. ⊙ = significantly different from controls at the 5% level or better. Experimental groups = 6 animals, control groups, 3–4 animals each.

Their bone calcium data did not show any significant loss of bone calcium. This may have been due to the fact that existing cycles in bone calcium were masked by summarizing all the data during the 4-week period. We have demonstrated the existence of cycles in bone calcium in rats exposed to 15% CO_2 for 7 days (Pasquale and Schaefer 1977). There is sufficient evidence in rats and guinea pigs exposed to different CO_2 concentrations that CO_2 uptake in bone is associated with calcium loss during specific phases of CO_2 binding in the bone.

The time course of CO_2 uptake in bones during exposure to 1% CO_2 has been determined by indirect titrimetry and the results have been reported elsewhere (Schaefer, Pasquale, and Messier 1976). The bicarbonate fraction of bone CO_2 showed two phases of significant increases during the first and sixth week of exposure; these increases coincided with periods of bone calcium and phosphorus loss (Fig. 2). Bone calcium mobilization leads to hypercalcemia (Fig. 2), which could cause kidney calcification just as it does in hypercalcemia produced by administration of parathyroid hormone (Cohn, Bowden, and Eller 1967). These authors demonstrated that the kidney is a target organ for parathyroid hormone action, because calcium accumulation occurred only in the kidneys and not in the liver or heart. Analysis of the calcium content of heart tissues from guinea pigs exposed up to 4 weeks to 1% CO_2 did not show any increase in calcium. These preliminary findings suggest that the kidney calcification seen during exposure to 1% CO_2 may involve the parathyroid hormone. Although ionized calcium showed only small increases that were not statistically significant during 4 weeks of exposure to 1% CO_2 (Table 2), this small rise may have stimulated the parathyroid gland. The fact that plasma calcium remains continuously elevated in animals exposed to 1% CO_2, independent of the two episodes of calcium flux out of the bone that occurred at one and six weeks,

TABLE 2
EFFECT OF EXPOSURE OF GUINEA PIGS FOR 4 WEEKS TO 1% CO_2 ON ACID-BASE PARAMETERS AND IONIZED CALCIUM

	H^+, nmol/liter	Pa_{CO_2}, mmHg	Ionized Calcium, mg %
		Control	
$\bar{X}$	37.1	35.9	5.0
SE	0.9	2.3	0.10
n	10.0	10.0	10.0
		Exposure to 1% CO_2	
1 Day, $\bar{X}$	39.4	37.6	5.1
SE	1.9	2.6	0.11
n	6.0	6.0	6.00
1 Week, $\bar{X}$	40.9*	37.8	5.2
SE	1.3	1.7	0.10
n	8.0	8.0	8.0
2 Weeks, $\bar{X}$	40.7*	37.2	5.0
SE	1.6	2.4	0.09
n	5.0	5.0	5.0
3 Weeks, $\bar{X}$	41.7*	38.9	5.3
SE	1.6	0.11	0.13
n	5.0	5.0	5.0
4 Weeks, $\bar{X}$	40.9*	38.9	5.0
SE	1.8	1.9	0.13
n	6.0	6.0	6.0

* = Significant at 0.05 level.

also seems to support the notion that a stimulation of the parathyroid gland existed throughout the 1% CO_2 exposure. Moreover, PTH may have been the cause of the acidosis observed during prolonged exposure to 1% CO_2. The decreased standard bicarbonate level indicates a failure of the kidney to reabsorb bicarbonate. PTH has been found to decrease bicarbonate reabsorption and to produce a systemic acidosis (Crumb, Martinez-Muldonado, Eknoyan, and Suki 1974). The same authors reported that hypercalcemia causes an increased bicarbonate reabsorption, but this effect can be suppressed by increased levels of PTH. The fact that elevated blood calcium levels found during prolonged exposure to 1% CO_2 failed to produce an increase in bicarbonate reabsorption gives further support to the notion of increased PTH activity under these conditions.

These findings, which suggest that small elevations of P_{CO_2} produced by prolonged exposure to 1% CO_2 may cause sustained increases in PTH activity and lead to a systemic acidosis, may increase our understanding of the mechanisms underlying the development of the systemic acidosis associated with primary and secondary hyperparathyroidism seen in the clinic (Muldowney, Freaney, and McGeeney 1968; Siddiqui and Wilson 1972).

In studies of magnesium deficiency, a sudden increase in plasma phosphate has been named as a factor precipitating kidney calcification (Hamuro et al. 1970). The initial increase in

TABLE 3
EFFECT OF PROLONGED EXPOSURE TO 15% CO_2 IN 21% O_2, BALANCE N_2 ON ACID-BASE PARAMETERS AND KIDNEY CALCIUM CONTENT

	pH	P_{CO_2}, mmHg	Kidney Calcium, mg/g (wet weight)	Blood Calcium, mg/100 ml
		Control		
$\bar{X}$	7.369	37.7	0.115	11.25
SE	0.013	0.9	0.009	0.08
n	8.0	8.0	8.0	8.0
		Exposure to 15% CO_2		
1 Hour, $\bar{X}$	7.078*	106.8*		—
SE	0.022	2.18		
n	6.0	6.0		
6 Hours, $\bar{X}$	7.085*	110.2*	0.124	11.75*
SE	0.025	2.1	0.002	0.08
n	6.0	6.0	6.0	6.0
1 Day, $\bar{X}$	7.088*	104.5*	0.125	11.78*
SE	0.047	5.8	0.008	0.08
n	6.0	6.0	6.0	6.0
3 Days, $\bar{X}$	7.178*	122.0	0.135*	12.44*
SE	0.021	8.9	0.002	0.08
n	6.0	6.0	6.0	6.0
7 Days, $\bar{X}$	7.230*	123.0*	0.169*	13.30*
SE	0.012	9.0	0.010	0.05
n	6.0	6.0	6.0	6.0

pH and P_{CO_2} data were corrected to body temperature; *significantly different from controls at the 5% level or better.

plasma phosphate occurred after the first day of exposure. Bone phosphorus data were not obtained at this point and the basis of the rise in plasma phosphorus on Day 1 of the exposure to 1% CO_2 cannot be explained on the basis of the available data. After one week of exposure to 1% CO_2, plasma phosphate was still elevated, which correlated with bone phosphorus loss. The early rise in plasma phosphorus might have contributed to kidney calcification, and the initial precipitation of calcification might have been sufficient to maintain the process. It has been pointed out that once kidney calcification has been started, mineralization becomes progressive because the solubility product for calcium is always exceeded in the serum (Ryke 1973).

Previous histopathological investigations of the kidney under conditions of chronic hypercapnia were carried out under higher CO_2 concentrations (3% CO_2 and above) without concomitant analysis of kidney calcium content (Meesen 1948; Zinck 1949; Schaefer et al. 1961). Since the most extensive histopathological investigations of kidney calcification were made in guinea pigs during exposure to 15% CO_2 (Schaefer et al. 1961), which served as a reference point, we repeated this experiment and measured kidney calcium content; these data are presented in Table 3. After 3 days of exposure, kidney calcium content was significantly elevated, and it rose by 47% on the 7th day of exposure to 15% CO_2. Blood calcium was

significantly increased throughout the exposure period. These data underline the significance of kidney calcification in chronic hypercapnia caused by different levels of CO_2 concentration.

To put the findings reported here into perspective, it has to be emphasized that exposure of guinea pigs and rats to CO_2 concentrations ranging from 1–20% for periods up to 6 months (1.5% CO_2) and 72 days (15% CO_2) did not produce any severe tissue damage and post-mortem examination of the animals did not show any untoward effects. As a matter of fact, there is a paucity of histopathological effects in chronic CO_2 poisoning, which contrasts favorably with the histopathology observed in other environmental stress conditions, e.g., chronic hypoxia (Schaefer, Niemoeller, Messier, Heyder, and Spencer 1971). The problems encountered in chronic CO_2 poisoning are primarily related to the adaptation of acid-base regulation.

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Schaefer, K. E., S. M. Pasquale, A. A. Messier, and H. Niemoeller. 1979. Calcification rénale provoquée par CO_2. Undersea Biomed. Res. Sub. Suppl.: S143–S153.—Des foyers de calcification rénale sont observés chez des cobayes après 42 jours d'exposition à 1,5% CO_2 (21% O_2, reste N_2), et chez des rats exposés pendant 91 jours. L'incidence des anomalies est en rapport directe avec la durée de l'exposition. La calcification se localise surtout au niveau des tubules du cortex rénal. Les cobayes sont exposés pendant des périodes jusqu'à 6 semaines et sont ensuite sacrifiés avec des témoins de la même portée. Chez les animaux exposés, P_{CO_2} artériel est augmenté de 3–4 mmHg, et les ions d'hydrogène de 4 nmol/liter. Le bicarbonate standard est réduit de 1–1,5 mmol, ce qui trahit une réabsorption rénale de bicarbonate (HCO_3) compromise. Ceci fait travailler d'avantage le système de tamponnage osseux, et parait provoquer la mobilisation du calcium et du phosphore osseux. Des baisses cycliques des deux minéraux sont observées après une et six semaines, et provoquent des hypercalcémies et des hyperphosphatémies cycliques. Une augmentation significative du calcium rénal est observée après deux semaines d'exposition; la concentration ne diminue pas pendant les semaines suivantes. Ces résultats montrent que la minéralization rénale ne dépend pas des variations calcémiques, une fois les processus rénaux commencés. Une augmentation immédiate de la phosphatémie après un jour d'exposition aurait pu jouer un role décisif. Les augmentations menues mais constantes de calcium ionisé pendant quatre semaines d'exposition à CO_2 (1%) peuvent avoir abouti à une stimulation des parathyroides, qui aurait maintenue une calcémie accrue pendant la période d'exposition; cette calcémie serait indépendente de la calcémie élevée associée à la libération du calcium des os.

toxicité chronique de CO_2
métabolisme du calcium
métabolisme du phosphore
acidose métabolique
calcification rénale

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